Congenital insensitivity to pain with anhydrosis in a Malaysian family: a genetic analysis

A Shalimar, I Sharaf
Department of Orthopaedics and Traumatology, Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia

I Farah Wahida
Tissue Engineering Laboratory, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia

BHI Ruszymah
Department of Physiology, Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia

ABSTRACT

A Malaysian family with congenital insensitivity to pain with anhydrosis was diagnosed based on clinical symptoms of chronic ulcers, joint deformities, malunited fractures, anhydrosis, and learning disabilities. We detected a compound heterozygous mutation in exon 16: V709L from the mother and G718S from the father. Two novel mutations were identified: at amino acid 709, a change of G to C at nucleotide 2209 (~2209G→C) causing a valine to leucine substitution (V709L), and at amino acid 718, a change of G to A at nucleotide 2236 (~2236G→A) causing a glycine to serine substitution (G718S). Polymorphisms identified were at nucleotides ~2113G→C and ~2176T→C.

Key words: genetic screening; hereditary sensory and autonomic neuropathies; mutation

INTRODUCTION

Congenital insensitivity to pain with anhydrosis (CIPA) is a rare disorder caused by a defect in the neurotrophin signal transduction system and is classified under hereditary sensory and autonomic neuropathies (HSAN).1 Proprioception, temperature sensitivity, and vibratory sensation may be affected along with insensitivity to pain, with some patients having wider systemic problems.2 CIPA is categorised as HSAN type IV under the Dyck classification1: type I is relatively mild, mainly affects the lower limbs and manifests in the second to fourth decade. Type II is more severe and presents in infancy. Type III is familial dysautonomia or Riley-Day syndrome. It is multisystemic and affects mainly Ashkenazi Jews. Type IV features insensitivity to pain, heat intolerance, and mental deficiency. Type V affects nociception selectively.

Diagnosis of CIPA is based on the clinical presentation, which is characterised by an infant history of recurrent fever, dermatological problems,
insensitivity to pain, anhydrosis, multiple infections, fractures and malunion, avascular necrosis, Charcot arthropathies, and joint dislocations. Self-mutilating behaviour and learning disabilities may also be evident. Skin and nerve biopsies, and a molecular deoxyribonucleic acid (DNA) analysis may confirm the diagnosis.\textsuperscript{1,3}

The neuronal system has neuronal differentiation and survival molecules that include neurotrophic molecules. Neurotrophins bind to tyrosine kinase receptors: TrkA, TrkB, or TrkC and a common neurotrophin receptor p75 (p75NTR) that has no tyrosine kinase domain. Nerve growth factor (NGF) is a neurotrophin that binds specifically to TrkA. In response to NGF, the receptor tyrosine kinase is autophosphorylated and activates intracellular signal transduction pathways. Thus, NGF plays an important role in the survival of selected populations of nociceptive sensory and autonomic sympathetic neurons. Mutations in the TRKA gene have been found to cause the CIPA syndrome.\textsuperscript{3}

The human TRKA gene is located on chromosome 1q21-22. The TRKA gene is divided into 17 exons and 16 introns, a sequence spanning at least 23 kb, coding for a protein of 790 or 796 amino acid residues. The TRKA gene encodes for the high-affinity TrkA, an essential part of the pain pathway.

Loss-of-function mutations in the TRKA gene have been derived from people with CIPA. At least 25 mutations have been found, including Q9X, N67fs, L93fs, G548fs (common Japanese founder mutation), and R596X.\textsuperscript{3-9} We aimed to further identify the abnormal gene and understand gene function controlling nociception and sensory neurons.

**CASE REPORT**

This study was approved by the research and ethical committee at our institution. The index family was first reported in 1999,\textsuperscript{10} when a child who presented with suspected ectodermal dysplasia, was shown to have hypotrophic sweat glands and skin structures after a skin biopsy. Her parents were asymptomatic and non-consanguineous, and had an asymptomatic eldest son and 3 symptomatic children (2 daughters and one youngest son) with varying severity—the oldest being least affected and the youngest worst affected (Fig. 1). They presented with phenotypes of CIPA: insensitivity to pain, chronic eczematous skin, non-healing ulcers, sparse hair, malunion, hyperlaxed and deformed joints (Fig. 2), and learning disabilities. They required mobility aids, especially the youngest who crawled on the floor. No other family members or earlier generations were known to be affected.

Genomic DNA was prepared from peripheral blood lymphocytes using standard polymerase chain reactions (PCR). The primer sequence for exons 1 to 17 were obtained.\textsuperscript{11} Mutations were detected by comparing published DNA and genomic sequences for the normal TRKA gene. We detected a compound heterozygous mutation in exon 16: V709L from the mother and G718S from the father. At amino acid 709, there was a change from G to C at nucleotide 2209 (~2209G\textsuperscript{C}) causing a valine to leucine substitution, seen in the mother, 2 daughters, and youngest son (Figs. 3a and 3b). At amino acid 718, there was a change from G to A at nucleotide 2236 (~2236G\textsuperscript{A}) causing a glycine to serine substitution, seen in the father and all children (Figs. 3c and 3d). The mother

![Figure 1](image_url)  
* Family tree of the index family.

* Mutated codon
and father were not affected as they carried only one mutation. Three of the children with both mutations expressed the disease phenotype. Therefore, it was unlikely to be a polymorphism.

Polymorphisms were detected at amino acid 677 of the mother with a change of G to C (~2113G→C) where valine remained as valine, and at amino acid 698 of the eldest son with a change of T to C (~2176T→C) where phenylalanine was substituted with leucine.

DISCUSSION

An Italian family has been shown to have a mutation at exon 16 where a homozygous G to A transition at nucleotide 2206 (~2206G→A) causes a glycine to serine substitution at amino acid 708. A ~2150C→T replacement was shown in Northern Israeli-Bedouin families resulting in a change of proline to leucine at amino acid 689. A polymorphism in exon 16 was seen in healthy controls demonstrating a ~2118A→G replacement but not changing the encoded amino acid. Our patients demonstrated 2 novel mutations on exon 16.

Although homozygous mutations are more common, compound heterozygous mutations have also been reported, including a combination of a M518V mutation and C1726 deletion, a combination of GN308fr and L213P, and a combination of L213P and G519fs. We did not undertake a single-strand confirmation polymorphism (SSCP) analysis to detect mutations, unlike other studies. SSCP is more appropriate for large population groups. It screens the population group before sequencing and detecting the exact mutations. Sequencing is the gold standard. The latest method of population analysis is the upgraded mutation detection or denaturing high performance liquid chromatography. An effective screening method detects ‘hotspots’ rather than analysing the entire gene. ‘Hotspots’ are regions where mutations are observed with greater frequency. Because of inherent instability, there is a tendency toward unequal crossing over or chemical predisposition to single nucleotide substitutions.

Molecular confirmation is not recommended as a means of diagnosing CIPA due to the large, unwieldy TRKA gene. The clinical presentation,
electromyography (EMG), and skin biopsy are sufficient for making the diagnosis. EMG may exclude other peripheral neuropathies, and the skin biopsy demonstrates absence of epidermal and sweat gland innervation. These provide a sensitive, reliable, and rapid means of diagnosing CIPA rather than DNA sequencing. Nonetheless, EMG and skin biopsies can be difficult to perform when patients, who are frequently children with learning disabilities, are not cooperative. Thus, the diagnosis of CIPA is usually based on clinical features.

Certain mutations are more common in certain populations. It may be appropriate to screen for the L213P mutations in people of northern European descent, while Japanese and Israeli-Bedouin patients should be screened for the R548fs and P615fs mutations respectively. We need to study other Malaysian families to determine whether they have the same mutations of V709L and G718S. Further studies are needed to discover how these mutations correlate with the pathogenesis of CIPA, including protein conformation studies on whether these mutations affect normal functioning of the neurotrophin signal transduction system.

ACKNOWLEDGEMENTS

We thank the index family members for their consent and cooperation. This study was funded by the Medical Research Centre of National University of Malaysia (Project Code FF-003-2004). We also thank Prof Yasuhiro Indo from the Department of Paediatrics, Kumamoto University School of Medicine, Kumamoto, Japan for his help in numbering the nucleotide sequence.

REFERENCES